Studies on Consequences of Genetically Modified Cotton on the Ecosystem

A Synopsis

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Title: "***Studies on Consequences of Genetically Modified Cotton on the Ecosystem***"

1) **Introduction**:

Presently about thirty agricultural Bt crops are industrially grown. The list of these crops includes maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), potato (*Solanum tuberasoum* L.), a particular variety of rape seed (*Brassica napus* L.), rice (*Oryza sativa* L.), mustard (*Brassica oleraceae* L.), groundnut (*Arachis hypogea* L.), brinjal (*Solanum melongena* L.), tobacco (*Nicotiana tabacum* L.), etc. the majority of transgenic maize species contain a gene encoding Cry I Ab protein that protects plants against a dangerous pest. The use of *Bt* crops widening throughout the world in 2004, these crops was 15.6 million ha. Including about 11.2 million ha for planting genetically modified maize in 2007 the area under Cry protein expressing transgenic crops increased almost twofold, the total area under Bt cotton in India and china was 10 million ha.(De Vries, J., Wackernagel1998). The Bt cotton targets only Bollworm insects (Vonzun, 2019). About 166 insects have been reported from India from cotton fields(Dhawan, 2016).

Bt- plants are genetically modified plants that contain (delta) endotoxin encoding genes of gram +ve aerobic spore forming bacterium *Bacillus thurengenesis*. The δ-endotoxins represents a class of numerous proteins with insecticidal action on larvae from various orders Cry I and Cry II B are toxic for the order Lepidoptera, Cry IIA, for Lepidoptera and Diptera. Mixtures composed of cells, spores, and parasporal (adjacent to spores) δ-endotoxin crystals became the pioneering preparations in biological fighting against agricultural pest. The first industrial Bt containing insecticide Sporein was developed in France in 1938. Since then this class of insecticides is thought to be among the best example of ecologically safe means of plant protection. The toxic effect of these insecticides on homeothermic animals was only observed at concentrations thousands times higher than those applied for treatment of agrocenoses (Dale, P.J., Clarke, B., Fontes. E.M.G.2002)

However there is reverse of the medal. Evidence is mounting that the use of Bt plants is always effective against pests and that growing of these plants can have a long term negative impact. The economic losses can be hardly evaluated thus so far. First, the Bt maize plants produce endotoxin in amounts 1500 to 2000 times higher than those spread during a

single application of *Bt* toxin containing chemical. Second, the decomposition of transgenic plants takes substantially longer time than that for genetically unmodified lines.

One of the important aspects of the risk assessment studies of genetically engineered (transgenic) plants is to study their impact on soil ecosystem. In this study it will be evaluated the impact of transgenic *Bt* cotton, expressing Cry1Ac protein, on soil microflora, nematodes, collembola and earthworms. Soil samples will be collected periodically, at different stages of crop growth, from the *Bt* cotton large-scale trial will be conducted at Botanical Garden Yeshwant Mahavidyalaya Nanded. Soil samples will be analyzed for level of Cry1Ac protein and the populations of culturable bacteria and fungi will be measured. Significant differences between *Bt* cotton, their non-*Bt* counterparts, and checks in terms of the total number of culturable bacteria and fungi will be observed. The quantity of Cry1Ac protein, if any in the soil will be measured by ELISA,

2. Review of Literature and Development in the subject

De Vries, J., Wackernagel1998studied the use of *Bt* crops widening throughout the world in 2004, these crops was 15.6 million ha. Including about 11.2 million ha for planting genetically modified maize in 2007 the area under Cry protein expressing transgenic crops increased almost twofold, the total area under Bt cotton in India and china was 10 million ha.

The toxic effect of these insecticides on homeothermic animals was only observed at concentrations thousands times higher than those applied for treatment of agrocenoses find out by **Dale, P.J., Clarke, B., Fontes. E.M.G.2002.** The transgenes incorporated in these crops were the modified *cry1Ab* in corn by **Carozzi and Koziel, 1997.**

The bacteria and fungi play an important role in the soil ecosystem as major decomposers of plant residues and releasing nutrients that sustain and stimulate plant growth in the process studied by **Lumsden R.D. 1981**.

Many plant associated bacterial and fungal species are susceptible to endotoxin produced by transgenic plants *in-vitro* by de **Vries *et al* 1999**, During K. 1994 and **Lottmant and Berg 2001**. The T_4 lysozyme expression of potato roots has the potential to change the structure of the microbial community in the soil fraction which is influenced by the plant roots (i.e. the rhizosphere) and subsequently to affect the quality or function of soil. Only a few studies of the effect of transgenic crop plants on the composition of microbial communities in the rhizosphere in the field condition by **Lottman et al 1999 and Siciliano and Germida 1999**, in the green house by **Di Giovanni et al 1999** have been published so far. Considering these facts the present project work has been proposed for study.

3. Objective:

- To study the diversity, composition and dynamics of the fungal community in the rhizosphere soil.
- To study the effect of *Bt*-cotton expressing cry1Ac and Non-*Bt* cottons on soil flora and fauna.
- To evaluate the effect of *Bt* cotton expressing the Cry1Ac protein on soil microflora.
- To determine the levels of Cry1Ac protein, if any, in soil during and after *Bt* cotton cultivation.

4)Methodology:

i) Collection of Seed Samples of Bt and non Bt cotton Plants.

ii) Field Preparation and field design.

iii) Soil sample collection before Plantation.

iv) Soil sample analysis before Plantation.

v) Determination of total fungal population.

vi) Culturing of isolated Fungi.

vii) Identification of bacterial species from soil samples.

viii) Identification of fungal species from soil samples.

ix) Collection of Earthworm Population.

x) Cross pollination in Bt and non- Bt cottons plants.

xi) Soil sample collection after plantation.

xii) Soil sample analysis after plantation.

5. Importance of study/ Society application

a) International status:

The year 1996 can be considered a landmark in agricultural biotechnology in general and crop protection in particular as four transgenic crops comprising three insect-resistant crops and a herbicide tolerant soybean, developed by Monsanto Company, received regulatory approvals and these were commercially grown and harvested for the first time in the USA. These approvals were preceded by about 14 years of intensive research and data generation that demonstrated these crops to be beneficial to farmers while, at the same time, being safe to humans, animals as well as other non-target beneficial organisms, plants and environment (Daniell and Muthukumar. 2001)

In 2004, transgenic crops were grown on 81.0 million hectares spread over 17 countries, including India, on six continents, marking a 47-fold increase in the area since their first commercialization in 1996. This increasing trend will continue in 2005 and in the coming decade. The dominant transgenic traits were insect resistance (IR) with *Bt* and herbicide tolerance (HT), either alone or both stacked. The principal transgenic crop was soybean with HR occupying 48.4 m ha followed by corn with IR and also HT plus IR on 19.3 m ha, cotton with IR and also HT plus IR on 9.0 m ha, and canola with HR on 4.3 m ha. The USA is the leading country in the commercial cultivation of transgenic crops, accounting for 59% (47.6 m ha) of the total 81 m ha followed by Argentina 20% (16.2 m ha), Canada 6% (5.4 m ha), Brazil 6% (5.0 m ha), China 5% (3.7 m ha), Paraguay 2% (1.2 m ha), India 1% (0.5 m ha) and South Africa 1% (0.5 m ha).

.All three insect-resistant crops were incorporated with genes that produce insecticidal proteins derived from the ubiquitous soil bacterium, *Bacillus thuringiensis*, popularly referred to as *Bt*. The proteins are expressed *in planta*. These crops were *Bt*-corn for protection against the notorious European corn borer - *Ostrinia nubilalis*, *Bt*-potato against the hardy Colorado potato beetle - *Leptinotarsa decemlineata,* and *Bt*-cotton against the dreaded cotton bollworm complex which includes the tobacco budworm - *Heliothis virescence*, bollworm - *Helicoverpa zea* and pink bollworm - *Pectinophora gossypiella*. The transgenes incorporated in these crops were the modified *cry1Ab* in corn (Carozzi and Koziel, 1997), modified *cry1Ac* in cotton (Perlak *et al.,* 1990) and modified *cry3Ab* in potato (Perlak *et al.,* 1993).

The gene EPSP synthase, also derived from a bacterium, was deployed in the herbicide tolerant soybean. In 1996, these crops were commercially cultivated not only in the USA, but also in Argentina and Canada on 1.7 million hectares. Although some products were formally approved for sale in limited areas prior to 1996, it was only in 1996 that farmers planted such large areas of biotech crops and continued to do so year after year in several countries. Thus, 2005 will be the 10th consecutive year of commercial planting of biotech crops on a significant scale. Meanwhile, herbicide tolerant canola as well as transgenic crops where both insect-resistant and herbicide tolerant genes were stacked in the same plant were also developed and commercialized. More countries started adopting transgenic crops. Further progress made in this area is described elsewhere in this article.

b) National Status :

In India, the area planted with *Bt*-cotton in 2002, the first year of introduction, was 29,415 ha. It increased to 86,240 ha in 2003 and to 530,800 ha in 2004. India recently made

its long-awaited entry into commercial agricultural biotechnology when the Genetic Engineering Approval Committee (GEAC), Ministry of Environment and Forests, Govt of India, at its 32nd meeting held in New Delhi on 26th March 2002 approved three *Bt*-cotton hybrids for commercial cultivation. This is a historic decision as *Bt*-cotton became the first transgenic crop to receive such an approval in India. These transgenic hybrids were developed by MAHYCO (Maharashtra Hybrid Seed Company Limited) in collaboration with Monsanto. These contain Monsanto's lepidopteron specific Bollgard*Bt* gene, *cry1Ac*, which offers protection against all the major species of Indian bollworms - *Helicoverpa armigera, Pectinophora gossypiella, Earias vittella and E. insulana*. These bollworms, especially *H. armigera*, have been responsible for heavy yield losses. Annual losses caused by bollworms alone are estimated at about US$ 300 million despite repeated spraying of insecticides (6 to 16 times or more for each crop). It is estimated that insecticides valued at $700 million are used on all crops annually in India, of which about 50% are used on the cotton crop alone. Since dependable alternative methods were not available, farmers had no option except to 'spray' or 'pray.' *Bt*-cotton has brought in a ray of hope (Barwale et al., 2004; Mohan and Manjunath, 2002; Manjunath, 2004).

The approval of *Bt*-cotton or Bollgard® (Bollgard® is the registered brand name for Monsanto's *Bt*-cotton) in India was preceded by about 500 field trials carried out in different agro-climatic regions between 1998 and 2001 to assess its efficacy against bollworms and the concomitant agronomic benefits. Experimental data on the bio-safety of *Bt*-cotton were generated by several public funded research institutions as per the direction of Department of Biotechnology (DBT), Ministry of Science & Technology, Government of India.

In addition to many trials conducted by MAHYCO as per DBT's guidelines and supervision, several *Bt*-cotton hybrids were also evaluated by ICAR (India Council of Agricultural Research) in multi-location field trials in 2001. These trials have indicated that *Bt*-cotton provided effective control of bollworms, requiring no or fewer application of chemical insecticides. The approval by GEAC was based on the strength of such scientific data. The *Bt*-cotton hybrids approved were MECH 12, MECH 162 and MECH 184. Following the approval, Mahyco-Monsanto Biotech Limited (MMB), a joint venture of Mahyco and Monsanto, which had only a limited stock of the *Bt* cotton seeds, sold these in six states of south and central India (Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Madhya Pradesh and Gujarat) to cover about 29,415 hectares . Thus, the first commercial planting of *Bt*-cotton occurred in the second half of 2002. Following its success, the area of *Bt*-cotton increased to 86,240 ha in 2003 and to 530,800 ha in 2004.

Realizing the potential of *Bt*-cotton, 19 other Indian seed companies have already become sub-licensees of MMB. These companies will introduce the *Bt*-gene into their own cotton hybrids developed for different agroclimatic regions and seek regulatory approvals. In fact, a hybrid namely RCH 2 developed by Rasi Seed Company, one of the major sub-licensees, has already completed the trials and received regulatory approval in 2004. At the same time, illegal *Bt*-cotton and spurious seeds have already found their entry into market. This issue is discussed separately (Manjunath, T. M. 2004).

c) Significance of the study:

1. Effect of transgenic plants on soil microflora will be commonly used to ensure uniform stand establishment to protect soil microflora.

   Effect of transgenic plants on soil microflora will be phenomenal success in to check the fertility of soil

6. Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work:-

1st year:-

1. Review of Literature
2. Selection of different varieties of Bt- cotton seeds
3. Soil analysis before plantation
4. Field Preparation and plantation
5. Collection of soil samples at different intervals from Bt and non- Bt cottons plants

2nd year:-

1. Isolation of fungi from Bt and non- Bt cottons plants
2. Culture of fungi identificationof Bt and non- Bt cottons plants
3. Identification of fungifrom Bt and non- Bt cottons plants
4. Collection of earthworm

3rd **year:-**

1. Sample collection after Plantation
2. Soil sampling analysis aftre Plantation
3. Cross pollination in Bt and non- Bt cottons plants
4. Submission of Thesis.

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